

a1 acid residues 1 to 607 (~~SEQ ID No. 11, Figure 1~~) (SEQ ID NO:11, Figure 1). In a further aspect the invention comprises a recombinant DNA plasmid that comprises the DNA sequence of the invention inserted into plasmid vectors and which can be used to drive the expression of the Δ Chy DNA polymerase in a host cell transformed with the plasmid. In a further aspect the invention includes a recombinant strain comprising the vector pDS56 carrying the Δ Chy DNA polymerase gene and designated p Δ_{2-225} AR₄. The ~~E. coli~~ *E. coli* strain XL1 carrying the plasmid p Δ_{2-225} AR₄ was deposited on the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascherorder Weg 1b, D-38124 Braunschweig DSM No. 11854 (BMTU 7307) is designated *E. coli* GA1.

page 7, line 6:

a2 Fig. Figure 2 shows the magnesium and manganese dependence of reverse transcriptase activity of Δ Chy ~~in dependence of magnesium and manganese salt~~.

page 8, line 23:

a3 *Carboxydotherrnus hydrogenofomans* DNA polymerase is the first DNA polymerase isolated from thermophilic eubacteria with a higher activity in the presence of magnesium ions than in the presence of manganese ions as shown in ~~figure~~ Figure 2. The magnesium dependence of reverse transcriptase activity ~~in dependence of magnesium is of advantage~~ advantageous since the DNA polymerase synthesize DNA with higher fidelity in the presence of magnesium than in the presence of manganese (Beckmann R.A. et al. (1985) *Biochemistry* 24, 5810-5817; Ricchetti M. and Buc H. (1993) *EMBO J.* 12, 387-396). Low fidelity DNA synthesis is likely to lead to mutated copies of the original template. In addition, Mn²⁺ ions have been implicated in an increased rate of RNA degradation, particularly at higher temperatures and this can cause the synthesis of shortened products in the reverse transcription reaction.

Page 9, line 5:

a4 The DNA sequence (~~SEQ ID No.:10~~) (SEQ ID NO:10) of Δ Chy polymerase and the derived amino acid sequence (~~SEQ ID No.:11~~) (SEQ ID NO:11) of the enzyme are shown in figure Figure 1. The molecular weight deduced from the sequence is 70.3 kDa, in SDS polyacrylamide gel electrophoresis however Δ Chy polymerase has an electrophoretic mobility of approximately 65 kDa.

Page 12, line 16:

a6
The production of a recombinant form of *Carboxydothemus hydrogenoformans* DNA polymerase generally includes the following steps: chromosomal DNA from *Carboxydothemus hydrogenoformans* is isolated by treating the cells with detergent e.g. SDS and a proteinase e.g. Proteinase K. The solution is extracted with phenol and chloroform and the DNA purified by precipitation with ethanol. The DNA is dissolved in Tris/EDTA buffer and the gene encoding the DNA polymerase is specifically amplified by the PCR technique using two mixed oligonucleotides (primer 1 and 2). These oligonucleotides, described by ~~SEQ ID No.: 1~~ SEQ ID NO:1 and ~~SEQ ID No.: 2~~, SEQ ID NO:2, were designed on the basis of conserved regions of family A DNA polymerases as published by Braithwaite D.K. and Ito J. (1993) *Nucl. Acids Res.* **21**, 787-802. The specifically amplified fragment is ligated into ~~an~~ a vector, preferably the pCRTMII vector (Invitrogen) and the sequence is determined by cycle-sequencing. Complete isolation of the coding region and the flanking sequences of the DNA polymerase gene can be performed by restriction fragmentation of the *Carboxydothemus hydrogenoformans* DNA with another restriction enzyme as in the first round of screening and by inverse PCR (Innis et al., (1990) PCR Protocols; Academic Press, Inc., 219-227). This can be accomplished with synthesized oligonucleotide primers binding at the outer DNA sequences of the gene part but in opposite orientation. These oligonucleotides described by ~~SEQ ID Nos. 3~~ SEQ ID NO:3 and 4, were designed on the basis of the sequences which were determined by sequencing of the first PCR product described above. As template DNA from *Carboxydothemus hydrogenoformans* is used which is cleaved by restriction digestion and circularized by contacting with T4 DNA ligase. To isolate the coding region of the entire polymerase gene, another PCR is performed using primers as shown in ~~SEQ ID Nos. 5~~ SEQ ID NO:5 and 6. The complete DNA polymerase gene is amplified directly from genomic DNA with primers suitable for introducing ends compatible with the linearized expression vector.

Page 13, line 11:

a7
~~SEQ ID No. 1:~~ SEQ ID NO:1:

Page 13, line 15:

a⁸ ~~SEQ ID No. 2:~~ SEQ ID NO:2:

Page 13, line 19:

a⁹ ~~SEQ ID No. 3:~~ SEQ ID NO:3:

Page 13, line 23:

a¹⁰ ~~SEQ ID NO. 4:~~ SEQ ID NO:4:

Page 13, line 27:

a¹¹ ~~SEQ ID NO. 5:~~ SEQ ID NO:5:

Page 14, line 2:

a¹² ~~SEQ ID NO. 6:~~ SEQ ID NO:6:

Page 15, line 1:

a¹³ ~~SEQ ID No. 7:~~ SEQ ID NO:7:

page 15, line 10:

a¹⁴ ~~SEQ ID NO. 8:~~ SEQ ID NO:8:

Page 15, line 14:

a¹⁵ The forward primer also contained an additional Nco I restriction site and additional 7 bases at the 5' end. Plasmid pDS56 DNA containing the polymerase-gene of *Carboxydotherrnus hydrogenoformans* at the Nco I/BamHI restriction sites was used as template for PCR. The PCR reaction was performed on the circular plasmid DNA pAR4. The fragment encoding the mutated *Carboxydotherrnus hydrogenoformans* DNA polymerase (Δ Chy) and the vector DNA were amplified as linear DNA by PCR using the Expand High Fidelity PCR System (Boehringer Mannheim) according to the supplier's specifications (~~Fig. 7~~): (Figure 7). The length of the gene encoding Δ Chy is 1821 bp.

Page 17, line 11:

a¹⁶ The amplification of target sequences from RNA may be performed to ~~proof~~ prove the presence of a particular sequence in the sample of nucleic acid to be analyzed or to clone a specific gene. Δ Chy DNA polymerase is very useful for these processes. Due to its 3'-5' exonuclease activity it is able to synthesize products with higher accuracy as than the reverse transcriptase of the state of the art.